(HLA match, n = 72; mismatch, n = 16), which contained a mean of 4.0 $\times 10^6$ CD34+ cells/kg (0.9–10.9 $\times$ 10^6 cells/kg) and 3.4 $\times$ 10^8 CD3+ cells/kg (1.1–9.0 $\times$ 10^8 CD3+ cells/kg). GVHD prophylaxis consisted of cyclosporine (n = 71) or tacrolimus (n = 17), with (n = 20) or without (n = 68) MTX, and 24 patients received additional ATG (5–10 mg/kg, Fresenius). The median values of ALC on days 0, 7, 14, 21, and 28 were, respectively, 68 (0–1460), 229 (0–1320), 675 (0–3185), 601 (0–3357), and 662 (0–2016). The median follow-up was 2 years (0.3–6.9). In the 36 patients whose ALC on day 0 was below 100, the proportion of patients who achieved complete donor T-cell chimerism (>90%) on day 28 was significantly higher than that of patients with ALC >100 (65% vs. 33%, p = 0.003). Patients whose ALC on day 0 was below 100 tended to show better OS than those with a value above 100 (69% vs. 63% at 2 y, p = 0.19). Furthermore, 50 patients whose ALC on day 28 was above 600 tended to show higher probabilities of grade II-IV aGVHD (54% vs. 34%, p = 0.051) and cGVHD (52% vs. 37%, p = 0.12) than the remaining 38 patients. However, the rate of positive CMV antigenemia (60% vs. 50%), relapse rate (42% vs. 37%), and OS (67% vs. 68%) at 2 y were not significantly different according to ALC on day 28. In conclusion, low ALC on day 0 may become a surrogate marker for effective host immune suppression resulting in higher donor T-cell chimerism after busulfan-based RIST, and early recovery of ALC on day 28 after RIST may predict GVHD, but not relapse or OS.

Opportunistic Infections by Time Period

Time	*Bacterial	Fungal	Viral	Pneumonia	Total
Admit-30 (n=52)	19 (2 lethal)	9 (0 lethal)	16 (2 lethal) (2 CMV; 9 HHV6; 4 BK; 1 Adeno)	3 (0 lethal)	47 (4 lethal)
+31–60 (n=48)	11 (0 lethal)	1 (0 lethal)	15 (2 lethal) (10 CMV; 1 HHV6; 3 BK; 1 Adeno)	0	27 (2 lethal)
+61–120 (n=41)	4 (0 lethal)	1 (0 lethal)	6 (1 lethal) (2 CMV; 1 Adeno; 1 RSV; 1 Influenza; 1 H. Zoster)	1 (0 lethal)	12 (1 lethal)
+121–180 (n=33)	3 (0 lethal)	0	4 (0 lethal) (1 CMV; 1 EBV; 1 RSV; 1 Influenza)	0	7 (0 lethal)
+180–1 yr (n=21)	1 (0 lethal)	0	3 (0 lethal) (1 CMV; 2 EBV)	0	4 (0 lethal)
Total	38 (2 lethal)	11 (0 lethal)	44 (5 lethal)	4 (0 lethal)	97 (7 lethal)

*C. Difficile infections were excluded.

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VIRAL INFECTIONS ARE THE GREATEST CAUSE OF INFECTIOUS MORTALITY AFTER CORD BLOOD (CB) TRANSPLANTATION (CBT) BUT THE MORTALITY RISK IS LIMITED TO THE FIRST 4 MONTHS

Sauter, C., Abboud, M., Hawke, R.M., Heller, G., Scaradavou, A., Barker, J.N. Memorial Sloan-Kettering Cancer Center, New York, NY

As CBT is associated with prolonged neutropenia the infection risk in the pre-engraftment period has received the most emphasis. However, aggressive antimicrobial prophylaxis can be effective in decreasing the lethality of bacterial and fungal infections whereas viral infections remain challenging. We reviewed the incidence of serious opportunistic infections (OI) in CBT recipients with the hypothesis that viral infections pose the biggest infectious lethality risk. 52 patients [median 38 years (range 3–66); 12 AML, 12 ALL, 28 lymphoma] received myeloablative (MA, n = 36) or non-myeloablative (NMA, n = 16) conditioning with cyclosporine-A/ mycophenolate mofetil and GCSF. Double unit grafts (4-6/6 HLA-A,B antigen, DRB1 allele matched to the patient) were used to enhance engraftment. Serious OI were defined as severe (required IV therapy/ admission), life-threatening, or fatal (caused or contributed to death). The cumulative incidence (CI) of sustained donor engraftment was 94% (95%CI:88–100) with the engrafting unit [1 6/6, 24 5/6, 23 4/6] having a median infused TNC dose of 2.2 $\times$ 10^7/kg (range 1.3–5.3). The median neutrophil recovery was 25 days (range 13–43) in MA and 11 days (range 7–36) in NMA recipients. The CI of grade II-IV acute graft-vs-host disease (GVHD) at day 100 was 40% (95%CI:27–53) and 6 patients have chronic GVHD to date. The day 180 transplant-related mortality was 24% (95%CI:13–35) and the 1 year overall survival is 64% (95%CI:52–80). The median CD4+ count/microL (359–1570) at days 60, 120, 180 and 1 year were 188, 272, 375 and 570, respectively. The median phytohemagglutinin (PHA) response (normal >92k) at these time points were 64k, 89k, 96k, and 140k, respectively. OIs by time-period are summarized below. "Pneumonias" responded to combined anti-bacterial/fungal therapy and the exact etiology was unknown. Viral infections accounted for the greatest infectious mortality. In contrast to bacterial/ fungal infections that diminished after day 30 viral infections continued to be a major problem in the post-engraftment period. However, subsequently their incidence decreased possibly related to immune recovery as suggested by a median CD4+ count >250/microL and PHA response >95% the lower limit of normal by day +120. In summary, the greatest risk for infection-related death is due to viral infections. Improved anti-viral agents and augmentation of anti-viral immunity should be a major priority in CBT.

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CHIMERISM DETECTION IN HEMATOPOIETIC CELL TRANSPLANT RECIPIENTS BY REAL-TIME QUANTITATIVE PCR (Q-PCR) VS. SHORT TANDEM REPEAT (STR) ANALYSIS

Senitzer, D.[1], Zhou, Y.[1], Gaidulis, L.[1], Lazaruk, K.[2] [1] City of Hope National Medical Center, Duarte, CA; [2] Celera, San Francisco, CA

STR is now being routinely used for chimerism detection in HCT. However, the sensitivity (<5%), multiplex PCR competition, and stutter band artifacts complicate quantitative analysis. We have evaluated a real-time quantitative PCR (Q-PCR)method for chimerism detection, and compared it with STR analyses. The ABI AmpFLSTR Identifiler Kit with 15 allelic markers was used for the STR chimerism study. Q-PCR procedure includes screening with 34 polymorphic markers to identify informative loci, followed by quantitation of the informative loci. The percentage of recipient chimerism is calculated based on the cycle threshold (Ct) value. To determine the accuracy of the Q-PCR method, fifty one peripheral blood, bone marrow, and cell subset samples from related and unrelated transplant cases were compared. The difference of the % recipient between Q-PCR and STR is listed in Table 1. To summarize, 8% of the Q-PCR data are exactly the same as the STR data; 86% have less than a 5% difference and 6% have less than a 10% difference. The sensitivity of the Q-PCR assay can reach 0.1% based on our serial dilution study. The specificity of the Q-PCR is 100% based on a 20 sample study. Both within run precision and between run precision of the Q-PCR are less than 10%. The variant amplification efficacy of each primer set in the multiplex STR contributes to the lower accuracy. Q-PCR eliminates the multiplex feature, and quantifies data with internal calibration. Q-PCR chimerism assay gives a high dynamic detection range from less than 0.1% to 100%. This advantage can be very useful in early detection of relapse. Ease of operation, and automated data analysis is a further advantage of Q-PCR, resulting in significant saving in labor costs . In conclusion, Q-PCR is a simple, fast, sensitive, and accurate assay for detection and quantitation of chimerism in HCT recipients.

Table 1. Chimerism Analysis By Q-PCR vs. STR

% Difference Between Q-PCR & STR	ASHI	Related	Unrelated	Total	%
0.0	2	0	2	4	8
0.1-5.0	11	11	22	44	86
5.1-10.0	1	1	1	3	6
Total	14	12	25	51	100